

# The role of macrophages in atherogenesis

Peter Libby\* and Steven K. Clinton†

\*Vascular Medicine and Atherosclerosis Unit, Cardiovascular Division, Department of Medicine, Brigham and Women's Hospital and †Dana-Farber Cancer Institute, Boston, Massachusetts, USA

Monocyte-derived macrophages and foam cells play a central role in atherogenesis. In vitro studies suggest that macrophages exhibit many functions relevant to lesion initiation, progression, and regression. Intimal foam cells function within an incompletely understood and complex network of cytokines, growth factors, and other mediators that vary in spatial and temporal distribution during lesion formation. Macrophages may modify lipoproteins to form derivatives that then modulate lesion formation. These cells also participate in local lipid metabolism within the atherosclerotic lesion by sequestering, processing, and exporting lipids. Macrophages participate in chronic immune and inflammatory aspects of plaque formation by elaborating a vast array of mediators, and by processing and presenting antigens to T-lymphocytes. The macrophage can elaborate both stimulators and inhibitors of smooth muscle cell migration and proliferation as well as regulate the elaboration of many constituents of the vascular matrix. Macrophages also express many of the enzymes involved in degrading matrix constituents. These cells may thereby play a central role in the remodeling of the extracellular matrix during smooth muscle cell migration, neovascularization, and plaque rupture. When a plaque is ruptured, macrophage-derived proteins that modulate blood coagulation and fibrinolysis participate in local clotting and contribute to lesion evolution as well as the transition from the chronic to the acute stages of atherosclerosis. The in vivo environment of the macrophage and foam cell is extremely complex, with multiple stimuli acting concomitantly. The information derived from in vitro studies of macrophage functions has yielded hypotheses that can now be tested *in vivo* using increasingly powerful experimental strategies.

Current Opinion in Lipidology 1993, 4:355-363

## Introduction

'If now ... we trace the development of the atheromatous condition a little farther back, we come — anteriorly to the period when the pulsatous matter is found in the seat of the atheroma — across a stage where nothing more is found than fatty degeneration in its ordinary form of granule cells...' [1].

For over a century, students of atherosclerosis have recognized the accumulation of foam cells (Virchow's granule cells in the above quotation) as a hallmark of atherosclerosis. The controversy over the origin of foam cells in atherosclerotic lesions yielded to the application of modern methodology for cell typing [2-6]. Although smooth muscle cells can also take up lipid, monocyte-de-

rived macrophages constitute the major source of foam cells in all stages of human and experimental atherosclerosis. This review will consider how recent work has expanded our understanding of the multiple mechanisms by which mononuclear phagocytes modulate atherogenesis.

## Recruitment of monocytes during atherogenesis

The recruitment and transformation of circulating monocytes into foam cells requires adhesion, transmigration, and accumulation of lipid, processes whose mechanisms and consequences are increasingly well understood. As early as the 1950s, Poole and Florey [7] recognized that

## Abbreviations

apo—apolipoprotein; IL—interleukin; MCP—monocyte chemoattractant protein; M-CSF—macrophage-colony stimulating factor; NO—nitric oxide radical; VCAM—vascular cell adhesion molecule.

© Current Science Ltd ISBN 1-85922-145-9 ISSN 0957-9672

355

For Copyright



BEST AVAILABLE COPY

monocyte adhesion to the luminal surface of the endothelium and penetration into the intima characterized the earliest phases of experimental atherogenesis in rabbits, a finding widely replicated in other species [8-11]. As atherosclerotic lesions progress, the expanded intima generally develops a rich network of newly formed microvessels [12]. These neovascular plexi tend to occur in regions rich in macrophages [13]. In the latter stages of lesion development, the luminal surface of these microvessels provides a large surface area for potential monocyte recruitment into the evolving lesion.

Recent work has provided much new information regarding the molecular mechanisms that underlie the 'patchy alteration in the endothelial surface' responsible for monocyte attachment postulated by Poole and Florey [7] nearly 4 decades ago. We now recognize a number of adhesion molecules expressed on the surface of endothelial cells in response to various stimuli that bind various classes of leukocytes [14]. These adhesion molecules fall into two general groups, members of the immunoglobulin gene superfamily and the selectins [15]. Members of the immunoglobulin G gene superfamily of potential relevance to atherogenesis include intercellular adhesion molecule-1, whose cognate ligands are the  $\beta_2$  integrins such as leukocyte function associated antigen-1 on the leukocyte surface. Vascular cell adhesion molecule (VCAM)-1 is another member of the immunoglobulin G superfamily expressed on various cell types, including vascular endothelium. VCAM-1 interacts with very late antigen-4, a  $\beta_1$  integrin expressed particularly on the surface of mononuclear leukocytes. Thus, VCAM-1 exhibits selectivity for monocytes and lymphocytes, precisely the types of leukocytes that accumulate within developing atheroma. Endothelial cells also show inducible expression of members of the selectin family of adhesion molecules such as E-selectin (formerly known as endothelial leukocyte adhesion molecule-1) or P-selectin (formerly known as GMP140 or as PADGEM), which interact preferentially with granulocytes rather than mononuclear cells. L-selectin (leukocyte adhesion molecule-1) expressed on the surface of leukocytes may interact with an as yet undefined ligand on endothelial cells.

The case of VCAM-1 merits particular attention in relation to monocyte recruitment during atherogenesis. Endothelial cells over nascent fatty streaks in rabbits with diet-induced or genetically determined hypercholesterolemia express VCAM-1 focally [16]. Endothelium in lesion-prone areas of the rabbit aorta express VCAM-1 as early as 1 week after initiating a hypercholesterolemic diet, before intimal macrophages accumulate [17]. Although these data support a possible role for VCAM-1 in monocyte recruitment, other adhesion molecules may also contribute to this process. It is noteworthy that in advanced human atheroma, microvascular endothelial cells express VCAM-1, supporting the possibility that these neovascular channels provide a portal for entry of mononuclear cells into the established lesion [18].

Once the monocyte attaches to the luminal endothelial cells, be they macro- or microvascular, chemoattractant

stimuli may promote their transendothelial migration and entry into the intima. The best studied candidate chemoattractant in this regard is monocyte chemoattractant protein (MCP)-1 [15,19]. This cytokine potently stimulates directed migration of monocytes *in vitro*. Vascular endothelial cells, smooth muscle cells, and leukocytes can inducibly express MCP-1 *in vitro* [20-25]. The regulation of MCP-1 in monocyte-derived cells is a matter of controversy [19]. Furthermore, *in situ* observations in human and experimental atherosclerotic lesions indicate expression of this chemoattractant molecule [26,27].

The mechanism of transendothelial penetration of the leukocytes *in vivo* remains a matter of speculation. Engagement of the cognate ligands of adhesion molecules may yield transmembrane signals which could activate the migratory machinery of the leukocyte. The precise links between hypercholesterolemia and other pro-atherogenic stimuli and the expression of monocyte adhesion molecules and chemoattractants by vascular cells *in vivo* remain incompletely defined. The possibility that oxidative modification of lipoproteins by vascular cells may initiate a cascade of early events is under investigation [22,28].

### Foam cell formation and lipid metabolism

Having entered the arterial intima, many mononuclear phagocytes differentiate into foam cells, a specialized type of macrophage. We now recognize that the accumulation of cholesteryl esters by macrophages occurs by the uptake of modified lipoprotein particles via specific membrane receptors. It was long appreciated that LDL suppresses the expression of its classical receptor [also known as the apolipoprotein (apo) B/E receptor] efficiently enough to prevent the accumulation of high concentrations of cholesteryl esters found in foam cells.

Brown and Goldstein [29] demonstrated the existence of a receptor for modified LDL using acetyl LDL as the model ligand. Krieger's group [30-33] subsequently cloned scavenger receptors I and II, members of a new family of transmembrane proteins, expressed by macrophages and other cells. The scavenger receptors can mediate lipid loading as even cholesterol-replete cells continue to express them. Macrophages within human atheroma express such scavenger receptors [31]. Circulating monocytes do not express the scavenger receptor which can be induced upon differentiation into macrophages *in vitro* [34]. Interestingly, macrophage-colony stimulating factor (M-CSF) can augment [34-36] and  $\gamma$ -interferon can suppress expression of the scavenger receptor [37]. *In vivo*, oxidative modification represents one plausible pathway for conferring upon lipoprotein particles the ability to bind scavenger receptors, a property modeled *in vitro* by acetylation. Recent evidence by Sparrow *et al* [38] suggests the existence of an additional receptor that binds highly oxidized LDL particles, and might provide an additional pathway for macrophage lipid accumulation. The molecular characterization of this putative 'oxidized LDL receptor' is incomplete at present.

The lesional macrophage not only accumulates lipid removed from the extracellular space but can process and possibly return lipids to the circulation [35,39]. It has been known for many years that mononuclear phagocytes can produce large quantities of apoE [29]. This apolipoprotein, an excellent ligand for the classical LDL receptor (the apoB,E receptor), may play a role in reverse cholesterol transport. Overexpression of apoE in transgenic mice can reduce diet-induced hypercholesterolemia [40]. Exogenous administration of apoE also inhibits lesion formation in hypercholesterolemic rabbits [41]. Genetic manipulation to inactivate apoE in mice render these usually atherosclerosis-resistant rodents susceptible to florid aortic foam cell lesion formation when fed lipid-enriched diets [42-44]. This enhanced atherogenesis accompanies a striking rise in 'remnant'-type lipoprotein particles in the blood of these apoE-deficient mice [43]. Factors that augment apoE expression in foam cells such as M-CSF may thus retard progression or promote regression of atherosclerosis [36,45]. ApoE may also modulate the immune response and aspects of tissue repair [46]. In additional apoE, macrophages can produce another apolipoprotein, serum amyloid A3, a component of certain HDL [47]. The content of serum amyloid A molecules derived from macrophages as well as liver may influence the metabolism of HDL particles.

Lesional macrophages can also express lipoxygenases, enzymes that may catalyze oxidative modification of intramural lipoproteins, furnishing products suitable as substrates for the scavenger uptake pathways and potentially capable of eliciting locally acting mediators [22,48,49]. However, oxidative modification of lipoproteins by cells may not require lipoxygenases [50]. Within human atheroma macrophages also produce lipoprotein lipase, an enzyme important in the remodeling of lipoprotein particles [51,52]. These recent observations broaden the traditional concept that this enzyme acts endovascularly at the luminal surface, and point to potential ongoing metabolism of lipoprotein constituents within the arterial wall itself during atherogenesis.

#### Activation and mediator production by macrophages

Mononuclear phagocytes, when appropriately stimulated, can produce many biologically active mediators that can influence virtually all aspects of atherogenesis [53]. A complete description of these mediators lies beyond the scope of this brief review. However, we shall illustrate the gamut of macrophage-derived mediators by highlighting selected classes grouped by function. Although many of these mediators may play pathogenic roles in atherosclerosis, some may exert protective functions during this process.

#### Regulators of smooth muscle proliferation

Smooth muscle cell migration and multiplication are likely to contribute to the formation of arterial hyperplastic diseases, including atherosclerosis. Macrophages provide one likely source of paracrine stimulators of smooth muscle cell proliferation. Macrophages can produce forms of platelet-derived growth factor [54,55], a family of mediators capable of stimulating smooth muscle proliferation *in vitro* and migration of smooth muscle cell *in vivo* [56]. Macrophages can also produce members of the epidermal growth factor family of smooth muscle mitogens. These include transforming growth factor- $\alpha$  [57] and a newly recognized heparin-binding epidermal growth factor-like molecule [58].

Macrophages can also produce basic fibroblast growth factor [59,60]. More recently we have found high levels of acidic fibroblast growth factor in association with macrophages in regions of neovascularization of advanced human atheroma [13]. *In vitro* studies [13] confirmed that macrophage-like cells (THP-1 cells exposed to phorbol esters for several weeks) could transcribe the gene for acidic fibroblast growth factor. Thus, the macrophage in lesions may produce both 'classical' forms of fibroblast growth factor, and may stimulate proliferation of endothelial cells as well as smooth muscle cells during different phases of atherogenesis.

Colony stimulating factors were originally described as factors that supported the survival, proliferation, or development, of various classes of bone-marrow-derived cells. M-CSF, a product of macrophages and other cells within atheromatous lesions [36,61], for example, is required for survival of mononuclear phagocytes, and renders them more responsive to mitogens such as interleukin (IL)-3 or granulocyte-macrophage-colony stimulating factor. Under some circumstances, smooth muscle cells can also express *c-fms*, the receptor for M-CSF [62-64]. Thus, in addition to altering a number of macrophage functions potentially relevant to atherosclerosis, M-CSF may modulate smooth muscle cell replication.

Interleukins such as IL-1  $\alpha$  and  $\beta$ , like the colony-stimulating factors, were initially thought to act on leukocyte targets exclusively. We now recognize cytokines such as IL-1 produced in large quantities by activated mononuclear phagocytes as candidates for signaling many pro-atherogenic functions of intrinsic vascular wall cells, including eliciting the production of endogenous growth factors that may amplify mitogenic effects [65-67].

#### Inhibitors of smooth muscle cell proliferation

Mononuclear phagocytes not only produce stimulators of smooth muscle cell proliferation, but can elaborate growth inhibitors as well. For example, macrophages can produce prostaglandins such as prostaglandin E<sub>2</sub>. Such prostanoids can inhibit proliferation of vascular

cells by raising intracellular levels of cyclic AMP [65]. Mononuclear phagocytes express an inducible form of the nitric oxide synthase, an enzyme that produces the nitric oxide radical (NO<sup>•</sup>) [68,69]. In high concentrations, NO exerts microbicidal actions important in host defenses against infectious agents. In the context of atherogenesis, NO may inhibit smooth muscle proliferation by increasing intracellular levels of cyclic GMP [70].

Certain cytokines and growth factors produced by macrophages can also inhibit cell proliferation. For example, tumor necrosis factor and IL-1, although they can promote proliferation of smooth muscle cells, inhibit the replication of endothelial cells. Transforming growth factor  $\beta$  can also inhibit smooth muscle proliferation under many experimental conditions *in vitro* [71-75]. Interestingly, transforming growth factor potently stimulates synthesis of interstitial forms of collagen by smooth muscle cells [76,77]. Thus, although it may limit the division of smooth muscle cells, it can promote accumulation of extracellular matrix during evolution of the atheromatous plaque [76].

### Matrix deposition and remodeling

Metabolism of the extracellular matrix has received surprisingly little attention in recent atherosclerosis research, although the matrix makes up the bulk of most complicated atherosclerotic lesions. Remodeling of the extracellular matrix doubtless contributes to lesion evolution by permitting migration of smooth muscle cells during intimal thickening and of endothelial cells during intralésional angiogenesis. Regional weakening in the integrity of the extracellular matrix in macrophage-rich regions seems to account for the transition from chronic to acute atherosclerosis in many cases of plaque rupture [78]. Macrophages produce in a regulatable fashion many of the enzymes considered important in remodeling of the vascular matrix. These enzymes represent a superfamily known as metalloproteinases because they depend upon a co-ordinated zinc atom for their catalytic activity. These enzymes share structural similarity, common catalytic mechanisms, and all exist as pro-enzymes or zymogens requiring limited proteolysis for activation.

Macrophages in atheroma can express forms of stromelysins, enzymes capable of activating the zymogens of the matrix metalloproteinase family [79]. Macrophages elaborate interstitial collagenase, important for degrading types I and III collagen, the most abundant forms of this matrix protein in atherosclerotic lesions [80,81]. Matrilysin also derives from mononuclear phagocytes [82]. These cells can also produce elastolytic activity required to hydrolyze elastin, another important protein of the vascular matrix [83-85].

Other matrix metalloproteinases produced by macrophages include gelatinases capable of further hydrolysis of collagen peptides released by the action of specific collagenases [86]. Certain gelatinases also can degrade the forms of collagen found in the basement

membrane in the vascular intima. Cytokines can modulate monocyte/macrophage expression of matrix metalloproteinases both positively and negatively [87,88]. In addition to producing these hydrolytic enzymes, macrophages can elaborate tissue inhibitors of metalloproteinases, endogenous antagonists of these enzymes [86]. Thus, macrophages within the atheroma possess a broad and tightly regulated complement of enzymes that can remodel the matrix of the lesion, and might contribute to its focal weakening at sites prone to plaque rupture.

### Macrophages as regulators of coagulation, thrombosis, and fibrinolysis

One of the consequences of plaque rupture is acute thrombosis. It is now recognized that control of hemostasis depends not only on soluble factors in the blood, but on regulators produced by cells of the arterial wall and by leukocytes. In this regard, mononuclear phagocytes in the atheroma can express a number of these key regulatory functions. Notably, lesional macrophages contain abundant tissue factor, an initiator of the so-called 'extrinsic' pathway of blood coagulation [89,90].

Macrophages can also produce an endogenous antagonist of plasminogen activator, plasminogen activator inhibitor-1 [91]. This protein can inhibit the endogenous urokinase or tissue-type plasminogen activators associated with macrophages and impede fibrinolysis, and thus stabilize any clot that may form in response to tissue factor. As in the case of several of the other regulatory systems described above, the macrophage has an entire complement of activities which can regulate local tendencies for blood clots to form and lyse [92].

### Heterogenous functions of lesional macrophage populations

Our appreciation of the subtleties of macrophage functions has increased substantially in the past few years. *In situ* analysis of many of the specific functions of macrophages indicates considerable heterogeneity in their expression within the atherosclerotic lesion. That is, even within the same atheroma, different macrophages may express many specific functions to a varying degree. Indeed, such heterogeneity appears the rule rather than the exception during atherogenesis.

For example, replication of macrophages occurs much more commonly in advanced atherosclerotic lesions than had been previously appreciated [93,94]. Yet, only a fraction of the mononuclear phagocytes within a lesion divide at any given time in the life history of that lesion. Similarly, only subpopulations of lesional macrophages appear to express a variety of other functions during any given 'slice in time' of atheroma sampling [13,27,52,90,95,96]. Some of this heterogeneity in expression

of specific macrophage functions may reflect the length of time in which a macrophage has resided within a lesion. The particular spectrum of macrophage foam-cell functions expressed by a given lesional cell may depend upon a local microenvironment of stimulatory and inhibitory cytokine signals. Alternatively, regional location of a macrophage may determine its functional state. For example, within the 'necrotic core' of advanced atherosclerotic plaques, necrobiosis of macrophages may occur. This cell death may result from accumulations of toxic concentrations of modified lipoprotein derivatives, of NO, or local depletion of the 'survival factor' M-CSF.

It is apparent that lesional macrophages can express certain functions episodically in response to systemic stimuli. For example, cytokines can often induce their own expression and expression of other cytokines within mononuclear phagocytes. It is conceivable that systemic cytokinemia, such as might be encountered by a systemic viral or bacterial infection, could evoke an 'echo' in macrophages resident within the atheroma, eliciting a wave of local induced cytokine expression [97]. In this way, macrophages resident in atheroma could provide an internal amplification loop for propagation of pro-inflammatory mediators in response to a systemic stimulus [98].

Inconsistencies in the literature caution against a simplistic view of activation of macrophage functions by modified lipoproteins. Some studies find that modified lipoproteins augment cytokine production or other pro-inflammatory functions of macrophages [99–102], whereas others find the opposite [103]. These apparently paradoxical results from reliable laboratories likely reflect different types of stimuli (highly versus 'minimally' modified LDL) and disparate assay cell types (mouse peritoneal macrophages versus human monocyte-derived macrophages). Such confusion highlights the variability of our experimental preparations, and the substantial differences between most mononuclear phagocytes studied *in vitro* and lesional foam cells, which themselves show considerable disparity in function as discussed. While cell culture studies provide a foundation for hypothesis generation, ultimately *in vivo* observations will furnish data to resolve these seeming contradictions.

## Conclusions

Macrophages play many roles during atherogenesis. Their functions intersect and influence diverse regulatory pathways including lipid metabolism, immune and inflammatory responses, growth control, matrix accumulation and remodeling, and thrombosis. We understand increasingly well the regulation of these various macrophage functions *in vitro*. Yet much of our extrapolation to atherogenesis in the intact animal, and in humans in particular, remains speculative. In the earlier stages of lesion initiation, increased adhesivity of monocytes to the endothelium by mechanisms currently understood in principle, provides a crucial initial step in the initiation of atherosclerosis. Formation of monocyte-derived foam cells is a hallmark of early atherogenesis. Sequestration of lipids within the

macrophage may represent an appropriate host defense to an abnormal accumulation of potentially toxic components of native or modified lipoproteins in the vessel wall. The elaboration of apoE by lesional macrophages may promote reverse cholesterol transport, another potentially salutatory action of macrophages during atherogenesis. By the same token, macrophages can produce oxygen radicals that might promote modification of lipoproteins. Such modification modulates the ability of lipoprotein constituents to elicit pro-inflammatory cytokines, growth factors, or other functions. The 'pulaceous' debris described by Virchow [1] may arise from necrobiosis of macrophages overburdened with toxic lipids and deprived of essential survival factors.

There is increasing appreciation of the chronic immune and inflammatory aspects of atherosclerotic lesion formation [104]. Macrophages are classical antigen-presenting cells, capable of processing antigens and presenting nominal antigens to T lymphocytes, initiating the cellular immune response. The presence of chronically stimulated T cells within lesions [105], and the widespread expression of class II histocompatibility antigens by lesional macrophages indicates that macrophages can participate decisively in the local immune response that occurs during atherogenesis.

Because the macrophage can elaborate both stimulators and inhibitors of smooth muscle cell proliferation, these cells may also participate in the control of replication of smooth muscle and other cells during atherogenesis. Many of the same factors that modulate the proliferative response also critically regulate the biosynthetic pathways that lead to matrix accumulation during lesion formation. Smooth muscle migration and plaque rupture during the evolution and complication of atheroma depend on matrix breakdown as well as synthesis. As macrophages can express many of the enzymes involved in degrading matrix constituents, these cells may not only control the synthesis but also the breakdown of matrix materials. The regulation of matrix remodeling, both temporally and spatially, may prove pivotal in the natural history of an atheroma.

The macrophage can also express many components of the machinery that regulate blood compatibility as expressed above. Once a plaque is ruptured, the coagulation proteins in the blood gain access to macrophage-derived tissue factor which can initiate local clotting. The consequent acute arterial thrombosis can lead to the dramatic acute clinical manifestations of atherosclerotic disease, including acute myocardial infarction or stroke.

Finally, we must continue to increase our appreciation of the subtlety of the activation programs of macrophages. We will not understand atherogenesis fully until we unravel the complexities of regulation of the various specific functional characteristics of these multipotent cells. Although *in vitro* studies provide inspiration, we must exercise considerable caution when extrapolating studies on macrophages and cell lines in culture to the behavior of foam cells within atheroma. The complexity, range, and heterogeneity in space and in time of macrophage functions present a worthy challenge for future investigation.

## Acknowledgments

This work is supported by a grant to Peter Libby from the National Institutes of Health HL34636 and by a Preventive Oncology Academic Award to Steven K. Clinton K-07 CA 01680. We thank Drs Zorina Galis, Michael Rosentfeld, Göran Hansson, and Robert Schaub for helpful discussions, and Ms Susan Hamer for editorial assistance in the preparation of this manuscript.

## References and recommending reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. VIRCHOW R: *Cellular Pathology*. John Churchill: London; 1858:342-366.
2. FOWLER S, SHIO H, HALEY NJ: Characterization of Lipid-Laden Aortic Cells from Cholesterol-fed Rabbits. IV. Investigation of Macrophage-Like Properties of Aortic Cell Populations. *Lab Invest* 1979, 41:372-378.
3. KLUFFEL DM: Identification of Foam Cells in Human Atherosclerotic Lesions as Macrophages Using Monoclonal Antibodies. *Arch Pathol Lab Med* 1985, 109:445-449.
4. AQEL NM, BALL RY, WALDMANN H, MITCHINSON MJ: Identification of Macrophages and Smooth Muscle Cells in Human Atherosclerosis Using Monoclonal Antibodies. *J Pathol* 1985, 146:197-204.
5. JONASSON I, HOLM J, SKALLI O, BONDJERS G, HANSSON GK: Regional Accumulations of T Cells, Macrophages, and Smooth Muscle Cells in the Human Atherosclerotic Plaque. *Arteriosclerosis* 1986, 6:131-138.
6. TSUKADA T, ROSENFELD M, ROSS R, GOWN AM: Immunocytochemical Analysis of Cellular Components in Lesions of Atherosclerosis in the Watanabe and Fat-Fed Rabbit Using Monoclonal Antibodies. *Arteriosclerosis* 1986, 6:601-613.
7. POOLE JCF, FLOREY HW: Changes in the Endothelium of the Aorta and the Behavior of Macrophages in Experimental Atheroma of Rabbits. *J Pathol Bact* 1958, 75:245-253.
8. GERRITY RG, GOSS JA, SOBY L: Control of Monocyte Recruitment by Chemotactic Factor(s) in Lesion-Prone Areas of Swine Aorta. *Arteriosclerosis* 1985, 5:55-66.
9. JORIS T, NUNNARI JJ, KROLIKOWSKI FJ, MAJNO G: Studies on the Pathogenesis of Atherosclerosis. I. Adhesion and Emigration of Mononuclear Cells in the Aorta of Hypercholesterolemic Rats. *Am J Pathol* 1983, 113:341-358.
10. FAGGIOTTO A, ROSS R, HARKER L: Studies of Hypercholesterolemia in the Nonhuman Primate. I. Changes that Lead to Fatty Streak Formation. *Arteriosclerosis* 1984, 4:323-340.
11. ROSENFELD ME, TSUKADA T, GOWN AM, ROSS R: Fatty Streak Initiation in Watanabe Heritable Hyperlipemic and Comparably Hypercholesterolemic Fat-Fed Rabbits. *Arteriosclerosis* 1987, 7:9-23.
12. BARGER A, BEEUWES IR, LAINEY L, SILVERMAN K: Hypothesis: Vasa Vasorum and Neovascularization of Human Coronary Arteries. *N Engl J Med* 1984, 310:175-177.
13. BROGI E, WINKLES J, UNDERWOOD R, CLINTON S, ALBERTS G, LIBBY P: Distinct Patterns of Expression of Fibroblast Growth Factors and their Receptors in Human Atheroma and Non-Atherosclerotic Arteries. Association of Acidic FGF with Plaque Microvessels and Macrophages. *J Clin Invest* 1994 (in press).
14. BEVILACQUA MP: Endothelial-Leukocyte Adhesion Molecules. *Annu Rev Immunol* 1993, 11:767-804.
15. CHARO IF: Monocyte-Endothelial Cell Interactions. *Curr Opin Lipidol* 1992, 3:335-343.
16. CYBULSKY MI, GIMBRONE MA JR: Endothelial Expression of a Mononuclear Leukocyte Adhesion Molecule during Atherogenesis. *Science* 1991, 251:788-791.
17. LI H, CYBULSKY MI, GIMBRONE MA JR, LIBBY P: An Atherogenic Diet Rapidly Induces VCAM-1, a Cytokine Regulatable Mononuclear Leukocyte Adhesion Molecule, in Rabbit Endothelium. *Arteriosclerosis Thromb* 1993, 13:197-204.
18. O'BRIEN K, ALLEN M, McDONALD T, CHAIT A, HARLAN J, FISHEIN D, MCCARTY J, FERGUSON M, HUDKINS K, BENJAMIN C, IORR R, ALPERS C: Vascular Cell Adhesion Molecule-1 is Expressed in Human Coronary Atherosclerotic Plaques: Implications for the Mode of Progression of Advanced Coronary Atherosclerosis. *J Clin Invest* 1993, 92:945-951.
19. ROLLINS BJ: JE/MCP-1: An Early-Response Gene Encodes a Monocyte-Specific Cytokine. *Cancer Cells* 1991, 3:517-524.
20. VALENTE AJ, FOWLER SR, SPRAGUE EA, KELLEY JL, SITTENRAM CA, SCHWARTZ CJ: Initial Characterization of a Peripheral Blood Mononuclear Cell Chemoattractant Derived from Cultured Arterial Smooth Muscle Cells. *Am J Pathol* 1984, 117:409-417.
21. TAUBMAN MB, ROLLINS BJ, NADAL-GINARD B: Expression of the JE Gene in Vascular Smooth Muscle Cells: Differential Effects of PDGF and Angiotensin II. *Circulation* 1989, 80:11-451.
22. CUSHING SD, BERLINER JA, VALENTE AJ, TERRITO MC, NAVAB M, PANHAMI F, GERRITY R, SCHWARTZ CJ, FOGELMAN AM: Minimally Modified Low Density Lipoprotein Induces Monocyte Chemotactic Protein 1 in Human Endothelial Cells and Smooth Muscle Cells. *Proc Natl Acad Sci U S A* 1990, 87:5134-5138.
23. ROLLINS BJ, YOSHIMURA T, LEONARD EJ, POBER JS: Cytokine-Activated Human Endothelial Cells Synthesize and Secrete a Monocyte Chemoattractant, MCP-1/JE. *Am J Pathol* 1990, 136:1229-1233.
24. WANG J, SICA A, PERI G, WALTER S, MARTIN-PADURA I, LIBBY P, CENKA M, LINDLEY I, COLOTTA F, MANTOVANI A: Expression of Monocyte Chemotactic Protein and Interleukin-8 by Cytokine-Activated Human Vascular Smooth Muscle Cells. *Arteriosclerosis* 1991, 11:1166-1174.
25. TAUBMAN MB, ROLLINS BJ, POON M, MARMUR J, GREEN RS, BERK BC, NADAL-GINARD B: JE mRNA Accumulates Rapidly in Aortic Injury and in Platelet-Derived Growth Factor-Stimulated Vascular Smooth Muscle Cells. *Circ Res* 1992, 70:314-325.

An authoritative review of a complex topic:

26. NEIKEN N, COUGHLIN S, GORDON D, WILCOX J: Monocyte Chemoattractant Protein-1 in Human Atherosclerotic Plaques. *J Clin Invest* 1991, 88:1121-1127.
27. YIA-HERTTUALA S, LIPTON RA, ROSENFELD ME, SARGOJA T, YOSHIMURA T, LEONARD EJ, WITZTUM JL, STEINBERG D: Expression of Monocyte Chemoattractant Protein 1 in Macrophage-Rich Areas of Human and Rabbit Atherosclerotic Lesions. *Proc Natl Acad Sci U S A* 1991, 88:5252-5256.
28. KLUM N, CYBULSKY MI, GIMBRONE MA JR: Lysophosphatidylcholine, a Component of Atherogenic Lipoproteins, Induces Mononuclear Leukocyte Adhesion Molecules in Cultured Human and Rabbit Arterial Endothelial Cells. *J Clin Invest* 1992, 90:1138-1144.
29. BROWN MS, GOLDSTEIN JL: Lipoprotein Metabolism in the Macrophage: Implications for Cholesterol Deposition in Atherosclerosis. *Annu Rev Biochem* 1983, 52:223-261.

30. KODAMA T, FREEMAN M, ROHRER L, ZABRECKY J, MATSUDAIRA P, KRIEGER M: Type I Macrophage Scavenger Receptor Contains Alpha-Helical and Collagen-Like Coiled Coils. *Nature* 1990, 343:531-535.
  31. MATSUMOTO A, NAITO M, ITAKURA H, IKEMOTO S, ASAKA H, HAYAKAWA I, KANAMORI H, ABURATANI H, TAKAKU F, SUZUKI H, ET AL: Human Macrophage Scavenger Receptors: Primary Structure, Expression, and Localization in Atherosclerotic Lesions. *Proc Natl Acad Sci U S A* 1990, 87:9133-9137.
  32. FREEMAN M, EKKEL Y, ROHRER L, PENMAN M, FREEDMAN NJ, CHISOLM GM, KRIEGER M: Expression of Type I and Type II Bovine Scavenger Receptors in Chinese Hamster Ovary Cells: Lipid Droplet Accumulation and Nonreciprocal Cross Competition by Acetylated and Oxidized Low Density Lipoprotein. *Proc Natl Acad Sci U S A* 1991, 88:4931-4935.
  33. KRIEGER M, ACTON S, ASHKENAS J, PEARSON A, PENMAN M, RESNICK D: Molecular Flypaper, Host Defense, and Atherosclerosis. Structure, Binding Properties, and Functions of Macrophage Scavenger Receptors. *J Biol Chem* 1993, 268:4569-4572.
- A current interpretive review.
34. SHIMANO H, YAMADA N, ISHIBASHI S, HARADA K, MATSUMOTO A, MORI N, INABA T, MOTYOYOSHI K, ITAKURA H, TAKAKU F: Human Monocyte Colony-Stimulating Factor Enhances the Clearance of Lipoproteins Containing Apolipoprotein B-100 via Both Low Density Lipoprotein Receptor-Dependent and -Independent Pathways in Rabbits. *J Biol Chem* 1990, 265:12869-12875.
  35. ISHIBASHI S, INABA T, SHIMANO H, HARADA K, INOUE I, MOKUNO H, MORI N, GOTODA T, TAKAKU F, YAMADA N: Monocyte Colony-Stimulating Factor Enhances Uptake and Degradation of Acetylated Low Density Lipoproteins and Cholesterol Esterification in Human Monocyte-Derived Macrophages. *J Biol Chem* 1990, 265:14109-14117.
  36. CLINTON S, UNDERWOOD R, SHERMAN M, KUFER D, LIBBY P: Macrophage-Colony Stimulating Factor Gene Expression in Vascular Cells and in Experimental and Human Atherosclerosis. *Am J Pathol* 1992, 140:301-316.
  37. GENG YJ, HANSSON GK: Interferon-Gamma Inhibits Scavenger Receptor Expression and Foam Cell Formation in Human Monocyte-Derived Macrophages. *J Clin Invest* 1992, 89:1322-1330.
  38. SPARKOW CP, PARTHASARATHY S, STEINBERG D: A Macrophage Receptor that Recognizes Oxidized Low Density Lipoprotein but not Acetylated Low Density Lipoprotein. *J Biol Chem* 1989, 264:2599-2604.
  39. YAMADA N, ISHIBASHI S, SHIMANO H, INABA T, GOTODA T, HARADA K, SHIMADA M, SHIOMI M, WATANABE Y, KAWAKAMI M, ET AL: Role of Monocyte Colony-Stimulating Factor in Foam Cell Generation. *Proc Soc Exp Biol Med* 1992, 200:240-244.
  40. SHIMANO H, YAMADA N, KATSHIKI M, SHIMADA M, GOTODA T, HARADA K, MURASE T, FUKAZAWA C, TAKAKU F, YAZAKI Y: Overexpression of Apolipoprotein E in Transgenic Mice: Marked Reduction in Plasma Lipoproteins Except High Density Lipoprotein and Resistance against Diet-Induced Hypercholesterolemia. *Proc Natl Acad Sci U S A* 1992, 89:1750-1754.
  41. YAMADA N, INOUE I, KAWAMURA M, HARADA K, WATANABE Y, SHIMANO H, GOTODA T, SHIMADA M, KOHIZAKI K, TSUKADA T, ET AL: Apolipoprotein E Prevents the Progression of Atherosclerosis in Watanabe Heritable Hyperlipidemic Rabbits. *J Clin Invest* 1992, 89:706-711.
  42. PIEDRAHITA JA, ZHANG SH, HAGAMAN JR, OLIVER PM, MAEDA N: Generation of Mice Carrying a Mutant Apolipoprotein E Gene Inactivated by Gene Targeting in Embryonic Stem Cells. *Proc Natl Acad Sci U S A* 1992, 89:4471-4475.
  43. PLUMP AS, SMITH JD, HAYEK T, AALTO-SETALA K, WALSH A, VERSTUYFT JG, RUBIN EM, BRESLOW JL: Severe Hypercholesterolemia and Atherosclerosis in Apolipoprotein E-Deficient Mice Created by Homologous Recombination in ES Cells. *Cell* 1992, 71:343-353.
- These experiments (and those in [44\*\*]) not only shed new light on the in vivo role of apoB, but provide a novel animal model for genetic study of atherogenesis.
44. ZHANG SH, REDDICK RL, PIEDRAHITA JA, MAEDA N: Spontaneous Hypercholesterolemia and Arterial Lesions in Mice Lacking Apolipoprotein E. *Science* 1992, 258:468-471.
- See [43\*\*].
45. INOUE I, INABA T, MOTYOYOSHI K, HARADA K, SHIMANO H, KAWAMURA M, GOTODA T, OKA T, SHIOMI M, WATANABE Y, ET AL: Macrophage Colony Stimulating Factor Prevents the Progression of Atherosclerosis in Watanabe Heritable Hyperlipidemic Rabbits. *Atherosclerosis* 1992, 93:245-254.
  46. MAHLEY RW: Apolipoprotein E: Cholesterol Transport Protein with Expanding Role in Cell Biology. *Science* 1988, 240:622-630.
  47. MEEK RL, ERINSEN N, BENNETT EP: Murine Serum Amyloid A3 Is a High Density Apolipoprotein and Is Secreted by Macrophages. *Proc Natl Acad Sci U S A* 1992, 89:7949-7952.
  48. YLA-HERTTUALA S, ROSENFELD ME, PARTHASARATHY S, GLASS CK, SIGAL E, WITZTUM JL, STEINBERG D: Colocalization of 15-Lipoxygenase mRNA and Protein with Epitopes of Oxidized Low Density Lipoprotein in Macrophage-Rich Areas of Atherosclerotic Lesions. *Proc Natl Acad Sci U S A* 1990, 87:6959-6963.
  49. LIAO F, BERLINER JA, MEHRABIAN M, NAVAB M, DEMER LL, LUSIS AJ, FOGELMAN AM: Minimally Modified Low Density Lipoprotein is Biologically Active In Vivo in Mice. *J Clin Invest* 1991, 87:2253-2257.
  50. SPARKOW CP, OLSZEWSKI J: Cellular Oxidative Modification of Low Density Lipoprotein Does not Require Lipoxygenases. *Proc Natl Acad Sci U S A* 1992, 89:128-131.
  51. YLA-HERTTUALA S, LIPTON BA, ROSENFELD ME, GOLDBERG JJ, STEINBERG D, WITZTUM JL: Macrophages and Smooth Muscle Cells Express Lipoprotein Lipase in Human and Rabbit Atherosclerotic Lesions. *Proc Natl Acad Sci U S A* 1991, 88:10143-10147.
  52. O'BRIEN KD, GORDON D, DEEB S, FERGUSON M, CHAIT A: Lipoprotein Lipase Is Synthesized by Macrophage-Derived Foam gC Cells in Human Coronary Atherosclerotic Plaques. *J Clin Invest* 1992, 89:1544-1550.
  53. NATHAN CF: Secretory Products of Macrophages. *J Clin Invest* 1987, 79:319-326.
  54. SHIMOKADO K, RAINES EW, MADTES DK, BARRETT TB, BENNETT EP, ROSS R: A Significant Part of Macrophage-Derived Growth Factor Consists of at Least Two Forms of PDGF. *Cell* 1985, 43:277-286.
  55. MARTINET Y, BITTERMAN PB, MORNEX JF, GRUTENDORST GR, MARTIN GR, CRISTAL RG: Activated Human Monocytes Express the c-Sis Proto-Oncogene and Release a Mediator Showing PDGF-Like Activity. *Nature* 1986, 319:158-160.
  56. FERNS G, RAINES E, SPRUGEL K, MOTANI A, REIDY M, ROSS R: Inhibition of Neointimal Smooth Muscle Accumulation after Angioplasty by an Antibody to PDGF. *Science* 1991, 253:1129-1132.
  57. MADTES DK, RAINES EW, SAKARIASSEN KS, ASSOIAN RK, SPORN MB, BELL GL, ROSS R: Induction of Transforming Growth Factor- $\alpha$  in Activated Human Alveolar Macrophages. *Cell* 1988, 53:285-293.
  58. HIGASHIYAMA S, ABRAHAM JA, MILLER J, FIDDES JC, KLAGSBRUN M: A Heparin-Binding Growth Factor Secreted by Macrophage-Like Cells that is Related to EGF. *Science* 1991, 251:936-939.
  59. BAIRD A, MOREMEUR P, BOHLEN P: Immunoreactive Fibroblast Growth Factor in Cells of Peritoneal Exudate Suggests its

- Identity with Macrophage-Derived Growth Factor. *Biochem Biophys Res Comm* 1985, 126:358-364.
60. JOSEPH-SILVERSTEIN J, MOSCATELLI D, RIFKIN BR: The Identification of bFGF in Adherent Elicited Murine Peritoneal Macrophages. *J Immunol Methods* 1988, 110:183-192.
  61. ROSENFEID M, YU-HERTTUALA S, LIPTON B, ORD V, WITZTUM J, STEINBERG D: Macrophage Colony-Stimulating Factor mRNA and Protein in Atherosclerotic Lesions of Rabbits and Humans. *Am J Pathol* 1992, 140:291-300.
  62. INABA T, YAMADA N, GOTODA T, SHIMANO H, SHIMADA M, MOMOMURA K, KADOWAKI T, MOTOTOYOSHI K, TSUKADA T, MORISAKI N ET AL: Expression of M-CSF Receptor Encoded by *c-fms* on Smooth Muscle Cells Derived from Arteriosclerotic lesion. *J Biol Chem* 1992, 267:5693-5699.
  63. INABA T, GOTODA T, SHIMANO H, SHIMADA M, HARADA K, KOZAKI K, WATANABE Y, HOH F, MOTOTOYOSHI K, YAZAKI Y ET AL: Platelet-Derived Growth Factor Induces *c-Fms* and Scavenger Receptor Genes in Vascular Smooth Muscle Cells. *J Biol Chem* 1992, 267:13107-13112.
  64. SHIMADA M, INABA T, SHIMANO H, GOTODA T, WATANABE Y, YAMAMOTO K, MOTOTOYOSHI K, YAZAKI Y, YAMADA N: Platelet-Derived Growth Factor BB-Dimer Suppresses the Expression of Macrophage Colony-Stimulating Factor in Human Vascular Smooth muscle cells. *J Biol Chem* 1992, 267:15455-15458.
  65. LIBBY P, WARNER SJC, FRIEDMAN GB: Interleukin-1: A Mitogen for Human Vascular Smooth Muscle Cells that Induces the Release of Growth-Inhibitory Prostanoids. *J Clin Invest* 1988, 88:487-498.
  66. RAINES EW, DOWER SK, ROSS R: Interleukin-1 Mitogenic Activity for Fibroblasts and Smooth Muscle Cells is Due to PDGF-AA. *Science* 1989, 243:393-396.
  67. GAY CG, WINKLES JA: Interleukin 1 Regulates Heparin-Binding Growth Factor 2 Gene Expression in Vascular Smooth Muscle cells. *Proc Natl Acad Sci U S A* 1991, 88:296-300.
  68. NATHAN C: Nitric Oxide as a Secretory Product of Mamalian Cells. *Faseb J* 1992, 6:3051-3064.
  69. LYONS CR, ORLOFF GJ, CUNNINGHAM JM: Molecular Cloning and Functional Expression of an Inducible Nitric Oxide Synthase from a Murine Macrophage Cell Line. *J Biol Chem* 1992, 267:6370-6374.
  70. GARG UC, HASSID A: Nitric Oxide-Generating Vasodilators and 8-Remo-Cyclic Guanosine Monophosphate Inhibit Mitogenesis and Proliferation of Cultured Rat Vascular Smooth Muscle Cells. *J Clin Invest* 1989, 83:1774-1777.
  71. MAJACK RA: Beta-Type Transforming Growth Factor Specifies Organizational Behavior in Vascular Smooth Muscle Cell Cultures. *J Cell Biol* 1987, 105:465-471.
  72. OWENS GK, GEISTERFER AA, YANG YW, KOMORIYA A: Transforming Growth Factor- $\beta$ -Induced Growth Inhibition and Cellular Hypertrophy in Cultured Vascular Smooth Muscle Cells. *J Cell Biol* 1988, 107:771-780.
  73. BATTAGAY EJ, RAINES EW, SEIFERT RA, BOWEN PDF, ROSS R: TGF- $\beta$  Induces Bimodal Proliferation of Connective Tissue Cells Via Complex Control of an Autocrine PDGF Loop. *Cell* 1990, 63:515-524.
  74. STOUFFER GA, OWENS GK: Angiotensin II-Induced Mitogenesis of Spontaneously Hypertensive Rat-Derived Cultured Smooth Muscle Cells Is Dependent on Autocrine Production of Transforming Growth Factor- $\beta$ . *Circ Res* 1992, 70:820-828.
  75. GIBBONS GH, PRATT RE, DZAU VJ: Vascular Smooth Muscle Cell Hypertrophy vs. Hyperplasia: Autocrine Transforming Growth Factor- $\beta$  1 Expression Determines Growth Response to Angiotensin II. *J Clin Invest* 1992, 90:456-461.
  76. LAU G, CHAN LM: Regulation of Extracellular Matrix RNA Levels in Cultured Smooth Muscle Cells: Relationship to Cellular Quiescence. *J Biol Chem* 1989, 264:1-6.
  77. AMENTO EP, EHSANI N, PALMER H, LIBBY P: Cytokines Positively and Negatively Regulate Interstitial Collagen Gene Expression in Human Vascular Smooth Muscle Cells. *Arteriosclerosis* 1991, 11:1223-1230.
  78. LENDON CL, DAVIES MJ, BORN GV, RICHARDSON PD: Atherosclerotic Plaque Caps are Locally Weakened When Macrophages Density Is Increased. *Atherosclerosis* 1991, 87:87-90.
  79. HENNEY AM, WAKELEY PR, DAVIES MJ, FOSTER K, HEMBRY R, MURPHY G, HUMPHRIES S: Localization of Stromelysin Gene Expression in Atherosclerotic Plaques by In Situ Hybridization. *Proc Natl Acad Sci U S A* 1991, 88:8154-8158.
  80. WELGUS HG, CAMPBELL IJ, CURY JD, EISEN AZ, SENIOR RM, WILHELM SM, GOLDBERG GI: Neutral Metalloproteinases Produced by Human Mononuclear Phagocytes: Enzyme Profile, Regulation, and Expression During Cellular Development. *J Clin Invest* 1990, 86:1496-1502.
  81. WELGUS HG, SENIOR RM, PARKS WC, KAHN AJ, LEY TJ, SHAPIRO SD, CAMPBELL EJ: Neutral Proteinase Expression by Human Mononuclear Phagocytes: A Prominent Role of Cellular Differentiation. *Matrix* 1992, 1:363-367.
  82. BUSNEK DF, ROSS FP, McDONNELL S, MURPHY G, MATRISIAN LM, WELGUS HG: The Matrix Metalloproteinase Matrilysin (PUMP) Is Expressed in Developing Human Mononuclear Phagocytes. *J Biol Chem* 1992, 267:9087-9092.
  83. BANTIA MJ, WEBB Z: Mouse Macrophage Elastase: Purification and Characterization as a Metalloproteinase. *Biochem J* 1981, 193:589-605.
  84. SHAPIRO SD, CAMPBELL EJ, WELGUS HG, SENIOR RM: Elastin Degradation by Mononuclear Phagocytes. *Ann N Y Acad Sci* 1991, 624:69-80.
  85. SHAPIRO SD, GRIFFIN GL, GILBERT DJ, JENKINS NA, COPELAND NG, WELGUS HG, SENIOR RM, LEY TJ: Molecular Cloning, Chromosomal Localization, and Bacterial Expression of a Murine Macrophage Metalloelastase. *J Biol Chem* 1992, 267:4664-4671.
  86. CAMPBELL EJ, CURY JD, SHAPIRO SD, GOLDBERG GI, WELGUS HG: Neutral Proteinases of Human Mononuclear Phagocytes: Cellular Differentiation Markedly Alters Cell Phenotype for Serine Proteinases, Metalloproteinases, and Tissue Inhibitor of Metalloproteinases. *J Immunol* 1991, 146:1286-1293.
  87. LACRAZ S, NICOD L, GALVE-DE-ROCHEMONTDX B, BAUMBERGER C, DAYER JM, WELGUS HG: Suppression of Metalloproteinase Biosynthesis in Human Alveolar Macrophages by Interleukin-4. *J Clin Invest* 1992, 90:382-388.
  88. CORCORAN ML, STETLER-STEVENS ON WG, BROWN PD, WAHL JM: Interleukin 4 Inhibition of Prostaglandin E2 Synthesis Blocks Interstitial Collagenase and 92-kDa Type IV Collagenase/gelatinase Production by Human Monocytes. *J Biol Chem* 1992, 267:515-519.
  89. DRAKE TA, MORRISSEY JH, EDGINGTON TS: Selective Cellular Expression of Tissue Factor in Human Tissues: Implications for Disorders of Hemostasis and Thrombosis. *Am J Pathol* 1989, 134:1087-1097.
  90. WILCOX JN, SMITH KM, SCHWARTZ SM, GORDON D: Localization of Tissue Factor in the Normal Vessel Wall and in the Atherosclerotic Plaque. *Proc Natl Acad Sci USA* 1989, 86:2839-2843.
  91. SCHWARTZ BS, BRADSLAW JD: Regulation of Plasminogen Activator Inhibitor mRNA Levels in Lipopolysaccharide-Stimulated Human Monocytes: Correlation with Production of the Protein. *J Biol Chem* 1992, 267:7089-7094.
  92. HAMILTON JA, VAIRO G, KNIGHT KR, COCKS BG: Activation and Proliferation Signals in Murine Macrophages. *Bio-*



- chemical Signals Controlling the Regulation of Macrophage Urokinase-Type Plasminogen Activator Activity by Colony-Stimulating Factors and Other Agents. *Blood* 1991, 77:616-627.
93. GORDON D, REILLY MA, BENDITT EP, SCHWARTZ SM: Cell Proliferation in Human Coronary Arteries. *Proc Natl Acad Sci USA* 1990, 87:4600-4604.
  94. ROSENFELD ME, ROSS R: Macrophage and Smooth Muscle Cell Proliferation in Atherosclerotic Lesions of WHHL and Comparably Hypercholesterolemic Fat-Fed Rabbits. *Arteriosclerosis* 1990, 10:680-687.
  95. WILCOX JN, SMITH KM, WILLIAMS LT, SCHWARTZ SM, GORDON D: Platelet-Derived Growth Factor mRNA Detection in Human Atherosclerotic Plaques by In Situ Hybridization. *J Clin Invest* 1988, 82:1134-1143.
  96. SALOMON RN, UNDERWOOD R, DOYLE MV, WANG A, LIBBY P: Increased Apolipoprotein E and *c-fms* Gene Expression Without Elevated Interleukin 1 or 6 levels Indicate Selective Activation of Macrophage Functions in Advanced Human Atheroma. *Proc Natl Acad Sci USA* 1992, 89:2814-2818.
  97. FLEET JC, CUNTON SK, SALOMON RN, LOPPNOW H, LIBBY P: Atherogenic Diets Enhance Endotoxin-Stimulated Interleukin-1 and Tumor Necrosis Factor Gene Expression in Rabbit Aortae. *J Nutrition* 1992, 122:294-305.
  98. LIBBY P, FLEET J, SALOMON RN, LI H, LOPPNOW H, CUNTON S: Possible Roles of Cytokines in Atherogenesis. In *Atherosclerosis IX*. Edited by Stein O, Eisenberg S, Stein Y. Tel Aviv, Israel: R&L Creative Communications; 1992:339-350.
  99. QUINN MT, PAKTHASARATHY S, FONG LG, STEINBERG D: Oxidatively Modified Low Density Lipoproteins: A Potential Role in Recruitment and Retention of Monocyte/Macrophages during Atherogenesis. *Proc Natl Acad Sci USA* 1987, 84:2995-2998.
  100. BARATH P, FISHBEN MC, CAO J, REFENSON J, HELFANT RH, FORRESTER JS: Detection and Localization of Tumor Necrosis Factor in Human Atheroma. *Am J Cardiol* 1990, 65:297-302.
  101. FROSTEGARD J, WU R, GISCORBE R, HOLM G, LEFVERT AK, NILSSON J: Induction of T-cell Activation by Oxidized Low Density Lipoprotein. *Arteriosclerosis Thromb* 1992, 12:461-467.
  102. STIKO RAHM A, HULTGARDH-NILSSON A, REGNSTRÖM J, HAMSTEN A, NILSSON J: Native and Oxidized LDL Enhances Production of PDGF AA and the Surface Expression of PDGF Receptors in Cultured Human Smooth Muscle Cells. *Arteriosclerosis Thromb* 1992, 12:1099-1109.
  103. HAMILTON TA, MA GP, CHISOLM GM: Oxidized Low Density Lipoprotein Suppresses the Expression of Tumor Necrosis Factor- $\alpha$  mRNA in Stimulated Murine Peritoneal Macrophages. *J Immunol* 1990, 144:2343-2350.
  104. LIBBY P: Inflammatory and Immune Mechanisms in Atherogenesis. In *Atherosclerosis Reviews*. Edited by Leaf A, Weber P. New York: Raven Press; 1990:79-89.
  105. STEMME S, HOLM J, HANSSON GK: T Lymphocytes in Human Atherosclerotic Plaques Are Memory Cells Expressing CD45RO and the Integrin VLA-1. *Arteriosclerosis Thromb* 1992, 12:206-211.
- New evidence supporting chronic immune stimulation during human atherogenesis.

Peter Libby, Vascular Medicine and Atherosclerosis Unit, Cardiovascular Division, Department of Medicine, Brigham and Women's Hospital, Longwood Medical Research Center, Room 307, 221 Longwood Avenue, Boston, MA 02115, USA.

**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

☒ **BLACK BORDERS**

☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**

☐ **FADED TEXT OR DRAWING**

☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**

☐ **SKEWED/SLANTED IMAGES**

☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**

☐ **GRAY SCALE DOCUMENTS**

☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**

☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**

☐ **OTHER:** \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**